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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application Serial No: 10/764,676

Peter Rohnert, et al. Examiner T.A. Solola

Filed January 26, 2004 Art Unit 1626

For: Drug Preparation Comprising α-Lipoic Acid, Ambroxol And/Or Inhibitors Of The Angiotensin-Converting Enzyme (ACE) And Its Use For The Treatment Of Neurodegenerative Diseases

37 C.F.R. 1.132 Declaration

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Dr. Frank Striggow, declare:

1. That I am presently a co-founder and Chief Executive Officer of KeyNeurotek AG. I studied Biochemistry at Martin-Luther-University Halle/Wittenberg from 1984 to 1989 and in 1994 received a Ph.D. in Biochemistry and Cell Biology at Otto-von-Guericke-University Magdeburg, Germany. Subsequently, I have performed post-doctoral training at University of Connecticut Health Center, Farmington, Connecticut, and Marine Biological Laboratory, Woods Hole, Massachusetts. I have been investigating intra- and intercellular mechanisms of neuronal degeneration and protection over the past decade and clinical drug development for CNS and autoimmune diseases (traumatic brain injuries, stroke, Alzheimer disease). I received the

innovation award of Saxony-Anhalt, Germany, in 2002 and 2004, and the Innovation Award of the German Industry in 2006.

2. That, as shown by the figures enclosed herewith, using the invention described in this patent application, a significant neuroprotective and therapeutic effect can be obtained.

In particular, the data in Figure 1 demonstrate the effectiveness of the invention in a clinically relevant in vivo global ischemia gerbil model. The data used to create this figure was obtained prior to the priority date for the present application. For this Figure, ESP01 stands for ambroxol, LS stands for alpha-lipoic acid and ESP02 stands for enalapril (a inhibitor of the angiotensin-converting enzyme).

To obtain the data for this figures, the animals were intraperitonealy injected with mixtures of ambroxol, α-lipoic acid (LS) and enalapril, and NaCl as a control. For NaCl (n=33); ambroxol + α-lipoic acid + enalapril (n=7); ambroxol + α-lipoic acid (n= 11); ambroxol alone (n=7); "Schein-OP" = no ischemia. Figure 1 shows the number of intact neurons in the CA1 hippocampus region. As can be seen, the combination of ambroxol + α-lipoic acid + enalapril, as well as the combination of ambroxol and α-lipoic acid, resulted in significant neuro protection. Therefore, these data demonstrate that the invention is effective in vivo, and that the administration of ambroxol (ESP01) does not result in neuro protection.

In addition, attached Figure 2, Figure 3 and Figure 4 further demonstrate a synergetic effect of the combination of ambroxol and its salts or its prodrugs and at least one inhibitor of the angiotensin-converting enzyme (ACE). The data used to produce this figure were obtained prior to the filing date of the present application. In particular, these data show the relative effects of the presently claimed compositions on neuronal damage by comparing combinations of:

- ambroxol + α-lipoic acid;
- ambroxol (alone);
- ambroxol + enalapril (ACE inhibitor); and
- ambroxol + α-lipoic acid + enalapril (ACE inhibitor).

The data were obtained as follows. Organotypic slices of the hippocampus were prepared and cultivated by means of standardized methods using Wistar rats (age: 7-8 d, weight: 12-20 g).

Hippocampi were prepared after decapitation of the test animals and cut into slices of 375µm by means of a McIlwain tissue chopper (The Mickle Laboratory Engineering Co., Guildford, Surrey, US). The freshly prepared slices were immediately transferred into ice cold HAME-01 Prep Medium with 2 mM L-Glutamin (pH 7,35 at 6°C, saturated with O_2). After a microscopic analysis of the quality of the slices, only optically acceptable slices were transferred to Anopore™ membranes (Ø 25 mm, size of pores 0.02 µm) from cell and tissue culture inserts (Nunc, Wiesbaden). In this procedure, 4-6 slices/membrane are cultivated. After receiving the slices, the inserts were set into Nunclon™ cell and tissue culture-6-well plates (Nunc, Wiesbaden). These contained 1.2 ml culture medium/well. The culture medium (pH 7.35 at 37°C) was composed as follows: 75 % HBME-21 Exec Medium (Company Cell Concepts), 25 % heat-inactivated horse serum (Gibco). During the cultivation the cultures were aerated with air at a 2.8 % CO_2 concentration. Every 2-3 days a medium exchange was executed. After 7-10 days in culture, the quality of the slices is examined by microscope in order to define slices of good quality for the subsequent experiment.

For testing ischemia (OGD, oxygen glucose deprivation) the inserts were set into Ringer's solution (pH 7.4, replacement of glucose by the same amount of Mannito) directly before the experiment. For inducing oxygen/glucose deprivation, the slices were incubated for 25 minutes in Ringer-Mannitol with simultaneous aeration with 95 % N_2/5 % CO_2. After the depletion of oxygen/glucose all slice cultures were cultivated for an additional 24 hours in the presence of glucose and oxygen. The quantification of the neuronal damage wes performed by propidium iodid coloring (PI). Because of the polar structure of the agent, it is held back by cell membranes of vital cells, while it penetrates into the intracellular space of damaged cells whose membrane integrity has suffered. In this process there is no fluorescent signal until the PI has penetrated into the cell nucleus and attached itself to double-stranded DNA; the intensity of the signal is proportional to the cellular damage. Subsequently, a CCD camera (Vosskühler GmbH, Osnabrück) mounted on a inverse fluorescence microscope (Nicon) was used to digitally record and save the transmission and fluorescence pictures of all individual slices. Lucia M software from Laboratory Imaging (Prag, Czech Republic) is used for analyzing the experiment. The neuronal cell damage within each organotypic slice of hippocampus is calculated as the area of PI fluorescence in proportion to the whole slice.

The graphs in Figures 2, 3 and 4 each show whisker boxes with bars showing that range in which 50 % of all experimental data are found. The numbers represent the degree of the damage in % of the tissue slices used, i.e., for example, at a value of 15, 15 % of the surface of the slices are damaged, i.e., 15 % of the cells died due to the treatment. Under the same experimental conditions, but when a composition of the invention is used, the damage is reduced, e.g., to 7.5 %, which corresponds to a neuroprotection of 50 %.

Thus, from the attached figures, it can be seen that the combination ambroxol + enalapril shows a clear and significant neuro protection in a clinically relevant model of global ischemia. The same is true for the combination of ambroxol + enalapril + alpha-lipoic acid. As is also demonstrated in the Figures, no neuro protective effect is observed after the singular administration of ambroxol or the combination of ambroxol + alpha-lipoic acid. The same is true for enalapril or alpha-lipoic acid administered individually. Further, the data in these figures clearly demonstrate that the combinations of "ambroxol + enalapril" and of "ambroxol + α-lipoic acid + enalapril" show a significantly better effect than, for example, the combination of "ambroxol + α-lipoic acid" or "ambroxol" alone. These are entirely unexpected results.

3. That all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 101 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued there from.

Respectfully submitted,

11.12.2006
Date

Frank Striggow, Ph.D.